

Bistability in the Chemical Master Equation for Dual Phosphorylation Cycles

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 (Dated: October 21, 2011)

Dual phospho/dephosphorylation cycles, as well as covalent enzymatic-catalyzed modifications of substrates are widely diffused within cellular systems and are crucial for the control of complex responses such as learning, memory and cellular fate determination. Despite the large body of deterministic studies and the increasing work aimed at elucidating the effect of noise in such systems, some aspects remain unclear. Here we study the stationary distribution provided by the two-dimensional Chemical Master Equation for a well-known model of a two step phospho/dephosphorylation cycle using the quasi-steady state approximation of enzymatic kinetics. Our aim is to analyze the role of fluctuations and the molecules distribution properties in the transition to a bistable regime. When detailed balance conditions are satisfied it is possible to compute equilibrium distributions in a closed and explicit form. When detailed balance is not satisfied, the stationary non-equilibrium state is strongly influenced by the chemical fluxes. In the last case, we show how the external field derived from the generation and recombination transition rates, can be decomposed by the Helmholtz theorem, into a conservative and a rotational (irreversible) part. Moreover, this decomposition, allows to compute the stationary distribution via a perturbative approach. For a finite number of molecules there exists diffusion dynamics in a macroscopic region of the state space where a relevant transition rate between the two critical points is observed. Further, the stationary distribution function can be approximated by the solution of a Fokker-Planck equation. We illustrate the theoretical results using several numerical simulations.

I. INTRODUCTION

One of the most important aspects of biological systems is their capacity to learn and memorize patterns and to adapt themselves to exogenous and endogenous stimuli by tuning signal transduction pathways activity. The mechanistic description of this behavior is typically depicted as a “switch” that can drive the cell fate to different stable states characterized by some observables such as levels of proteins, messengers, organelles or phenotypes [21]. The biochemical machinery of signal transduction pathways is largely based on enzymatic reactions, whose average kinetic can be described within the framework of chemical kinetics and enzyme reactions as pioneered by Michaelis and Menten [9, 17]. The steady state velocity equation accounts for the majority of known enzymatic reactions, and can be adjusted to the description of regulatory properties such as cooperativity, allostericity and activation/inhibition[10]. Theoretical interest in enzymatic reactions has never stopped since Michaelis-Menten’s work and has lead to new discoveries such as zero-order ultrasensitivity [1, 4]. Among various enzymatic processes, a wide and important class comprises the reversible addition and removal of phosphoric groups via phosphorylation and dephosphorylation reactions catalyzed by kinases and phosphatases. The phospho/dephosphorylation cycle (PdPC) is a reversible post-translational substrate modification that is

central to cellular signalling regulation and can play a key role in the switch phenomenon for several biological processes ([7, 15]). Dual PdPC’s are classified as homogeneous and heterogeneous based on the number of different kinases and phosphatases ([6]); the homogeneous has one kinase and one phosphatase, while the heterogeneous has two kinases and two phosphatases. Variants of homogeneous and heterogeneous dual PdPC’s may only have a non-specific phosphatase and two specific kinases [3] or, symmetrically, a non-specific kinase and two specific phosphatases. The PdPC with the non-specific phosphatase controls the phosphorylation state of AMPA receptors that mediates induction of Long Term Potentiation (LTP) and Long Term Depression (LTD) in vitro and in vivo[2, 3, 20]. Recently, several authors have reported bistability in homogeneous pPdPC [6, 12]) as well as those with a non-specific phosphatase and two different kinases [2]. The bistable behaviour of the homogeneous system is explained on the basis of a competition between the substrates for the enzymes. The majority of studies on biophysical analysis of phospho/dephosphorylation cycle have been performed in a averaged, deterministic framework based on Michaelis-Menten (MM) approach, using the steady state approximation. However, recently some authors [8] have pointed to the role of fluctuations in the dynamics of biochemical reactions. Indeed, in a single cell, the concentration of molecules (substrates and enzymes) can be low, and thus it is necessary to study the PdPC cycle within a stochastic framework. A “natural” way to cope with this problem is the so-called Chemical Master Equation (CME) approach [18], that realizes in an exact way the proba-

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bilistic dynamics of a finite number of molecules, and recovers the chemical kinetics of the Law of Mass Action, which yields the continuous Michaelis-Menten equation in the thermodynamic limit ($N \rightarrow \infty$), using the mean field approximation. In this paper we study a stochastic formulation of enzymatic cycles that has been extensively considered by several authors ([6, 12]). The deterministic descriptions of these models characterize the stability of fixed points and give a geometrical interpretation of the observed steady states, as the intersection of conic curves([12]). The stochastic description can in fact provide further information on the relative stability of the different steady states in terms of a stationary distribution. We propose a perturbative approach for computing the stationary solution out of the thermodynamics equilibrium. We also point out the role of currents in the transition from a mono-modal distribution to a bimodal distribution; this corresponds to bifurcation in the deterministic approach. The possibility that chemical fluxes control the distribution shape suggests a generic mechanism used by biochemical systems out of thermodynamic equilibrium to obtain a plastic behavior. Moreover, we show that at the bimodal transition there exists a diffusion region in the configuration space where a Fokker-Planck equation can be introduced to approximate the stationary solution. Analogous models have been previously studied [1, 13, 14] for single-step PdPC.

II. DUAL PHOSPHORYLATION/DEPHOSPHORYLATION ENZYMATICAL CYCLES

The process shown in Fig. (1) is a two-step chain of addition/removal reactions of chemical groups and may, in general, model important biological processes such as phenotype switching (ultrasensitivity) and chromatin modification by histone acetylation/deacetylation as well as phospho/dephosphorylation reactions. Without loss of generality, we perform a detailed study of the homogeneous phospho/dephosphorylation two-step cycles (PdPC cycles) where two enzymes drive phosphorylation and dephosphorylation respectively. Thus, there is a competition between the two cycles for the advancement of the respective reactions.

The deterministic Michaelis-Menten (MM) equations of the scheme (1) with the quasi-steady-state hypothesis reads:

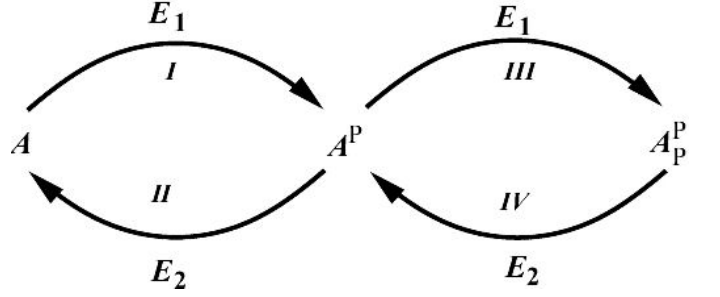


FIG. 1. Scheme of the double enzymatic cycle of addition/removal reactions of chemical groups via Michaelis-Menten kinetic equations as shown in eq (1) in the case of phosphoric groups.

$$\begin{aligned} \frac{dA}{dt} &= \frac{k_{M_4}v_{M_2}(1-A-A_P^P)}{k_{M_2}k_{M_4}+k_{M_4}(1-A-A_P^P)+k_{M_2}A_P^P} \\ &\quad - \frac{k_{M_3}v_{M_1}A}{k_{M_1}k_{M_3}+k_{M_1}(1-A-A_P^P)+k_{M_3}A} \\ \frac{dA_P^P}{dt} &= \frac{k_{M_1}v_{M_3}(1-A-A_P^P)}{k_{M_1}k_{M_3}+k_{M_1}(1-A-A_P^P)+k_{M_3}A} \\ &\quad - \frac{k_{M_2}v_{M_4}A_P^P}{k_{M_2}k_{M_4}+k_{M_4}(1-A-A_P^P)+k_{M_2}A_P^P} \quad (1) \end{aligned}$$

where A and A_P^P are the concentrations of the non-phosphorylated and double phosphorylated substrates, k_{M_i} denote the MM constants and v_{M_i} are the maximal reaction velocities ($i = 1, \dots, 4$). Let n_1 and n_2 denote the molecules number of the substrates A and A_P^P respectively, the corresponding CME for the probability distribution $\rho(n_1, n_2, t)$ is written

$$\begin{aligned} \frac{\partial \rho}{\partial t} &= g_1(n_1-1, n_2)\rho(n_1-1, n_2, t) - g_1(n_1, n_2)\rho(n_1, n_2, t) \\ &\quad + r_1(n_1+1, n_2)\rho(n_1+1, n_2, t) - r_1(n_1, n_2)\rho(n_1, n_2, t) \\ &\quad + g_2(n_1, n_2-1)\rho(n_1, n_2-1, t) - g_2(n_1, n_2)\rho(n_1, n_2, t) \\ &\quad + r_2(n_1, n_2+1)\rho(n_1, n_2+1, t) - r_2(n_1, n_2)\rho(n_1, n_2, t) \quad (2) \end{aligned}$$

where $g_j(n_1, n_2)$ and $r_j(n_1, n_2)$ are the generation and recombination terms respectively, defined as :

$$\begin{aligned} r_1(n_1, n_2) &= \frac{K_{M_3}v_{M_1}n_1}{K_{M_1}K_{M_3}+K_{M_1}(N_T-n_1-n_2)+K_{M_3}n_1} \\ g_1(n_1, n_2) &= \frac{K_{M_4}v_{M_2}(N_T-n_1-n_2)}{K_{M_2}K_{M_4}+K_{M_4}(N_T-n_1-n_2)+K_{M_2}n_2} \\ r_2(n_1, n_2) &= \frac{K_{M_2}v_{M_4}n_2}{K_{M_2}K_{M_4}+K_{M_4}(N_T-n_1-n_2)+K_{M_2}n_2} \\ g_2(n_1, n_2) &= \frac{K_{M_1}v_{M_3}(N_T-n_1-n_2)}{K_{M_1}K_{M_3}+K_{M_1}(N_T-n_1-n_2)+K_{M_3}n_1} \quad (3) \end{aligned}$$

N_T is the total number of molecules, and we have introduced scaled constants $K_M = N_T k_M$. The biochemical

meaning is that the enzyme quantities should scale as the total number of molecule N_T , to have a finite thermodynamic limit $N_T \rightarrow \infty$, in the transition rates (3). As it is known from the theory of one-step Markov processes, the CME (2) has a unique stationary solution $\rho_s(n_1, n_2)$ that describes the statistical properties of the system on a long time scale. The CME recovers the Mass Action-based MM equation (1) in the thermodynamic limit when the average field theory approach applies. Indeed it can be shown that the critical points of the stationary distribution for the CME can be approximately computed by the conditions (cfr. eq. (15))

$$g_1(n_1, n_2) = r_1(n_1 + 1, n_2) \quad g_2(n_1, n_2) = r_2(n_1, n_2 + 1) \quad (4)$$

whose solutions tend to the equilibrium points of the MM equation when the fluctuation effects are reduced in the thermodynamic limit as $O(1/\sqrt{N})$. As a consequence, one would expect that the probability distribution becomes singular, being concentrated at the fixed stable points of the equations (1), and that the transition rate among the stability regions of attractive points is negligible. However, when a phase transition occurs due to the bifurcation of the stable solution, fluctuations are relevant even for large N_T and the CME approach is necessary. In the next section we discuss the stationary distribution properties for the CME (2).

III. THE STATIONARY DISTRIBUTION

The stationary solution $\rho_s(n_1, n_2)$ of the CME (2) can be characterized by a discrete version of the zero divergence condition for the current vector \vec{J} components (see Appendix)

$$\begin{aligned} J_1^s &= g_1(n_1 - 1, n_2)\rho_s(n_1 - 1, n_2) - r_1(n_1, n_2)\rho_s(n_1, n_2) \\ J_2^s &= g_2(n_1, n_2 - 1)\rho_s(n_1, n_2 - 1) - r_2(n_1, n_2)\rho_s(n_1, n_2) \end{aligned} \quad (5)$$

and the CME r.h.s. reads

$$D_1 J_1^s(n_1, n_2) + D_2 J_2^s(n_1, n_2) = 0 \quad (6)$$

where we have introduced the difference operators

$$\begin{aligned} D_1 f(n_1, n_2) &= f(n_1 + 1, n_2) - f(n_1, n_2) \\ D_2 f(n_1, n_2) &= f(n_1, n_2 + 1) - f(n_1, n_2) \end{aligned} \quad (7)$$

Due to the commutative properties of the difference operators, the zero-divergence condition for the current is equivalent to the existence of a current potential $A(n_1, n_2)$ such that

$$\begin{aligned} J_1^s(n_1, n_2) &= D_2 A(n_1, n_2) \quad n_1 \geq 1 \\ J_2^s(n_1, n_2) &= -D_1 A(n_1, n_2) \quad n_2 \geq 1 \end{aligned} \quad (8)$$

We remark that the r.h.s. of eq. (8) is a discrete version of the curl operator. The potential difference $A(n'_1, n'_2) -$

$A(n_1, n_2)$ defines the chemical transport across any line connecting the states (n_1, n_2) and (n'_1, n'_2) . At the stationary state the net transport across any closed path is zero and we have no current source in the network. As discussed in [16, 18] we distinguish two cases: when the potential $A(n_1, n_2)$ is constant (the so called “detailed balance condition”) and the converse case corresponding to a non-equilibrium stationary state. In this case the stationary solution ρ_s is characterized by the condition $J_1 = J_2 = 0$ over all the states, whereas in the other case we have macroscopic chemical fluxes in the system. When detailed balance holds, simple algebraic manipulations (see Appendix) result in the following conditions for the stationary solutions

$$D_1 \ln \rho_s(n_1, n_2) = \ln a_1(n_1, n_2) \quad (9)$$

$$D_2 \ln \rho_s(n_1, n_2) = \ln a_2(n_1, n_2) \quad n_1 + n_2 < N_T \quad (10)$$

where the discrete drift vector field components a_i are defined by:

$$a_1(n_1, n_2) = \frac{g_1(n_1, n_2)}{r_1(n_1 + 1, n_2)} \quad a_2(n_1, n_2) = \frac{g_2(n_1, n_2)}{r_2(n_1, n_2 + 1)} \quad (11)$$

Equations (9) and (10) imply the existence of a potential $V(n_1, n_2)$ such that

$$\begin{aligned} \ln a_1(n_1, n_2) &= -D_1 V(n_1, n_2) \\ \ln a_2(n_1, n_2) &= -D_2 V(n_1, n_2) \end{aligned} \quad (12)$$

Using definition (3) it is possible to explicitly compute a set of parameter values for the PdPC cycle, that satisfy the detailed balance condition (12) according to the relations

$$2K_{M_1} = K_{M_2} = K_{M_3} = 2K_{M_4} \quad (13)$$

where the reaction velocities V_M are arbitrary. The stationary distribution is given by the Maxwell-Boltzmann distribution

$$\rho_s(n_1, n_2) = \exp(-V(n_1, n_2)) \quad (14)$$

where the potential $V(n_1, n_2)$ is computed by integrating equation (12) and choosing the initial value $V(0, 0)$ to normalize the distribution (14). Using a thermodynamical analogy, we can interpret the potential difference $V(n_1, n_2) - V(0, 0)$ as the chemical energy needed to reach the state (n_1, n_2) from the initial state $(0, 0)$. As a consequence, the vector field (11) represents the work for one-step transition along the n_1 or n_2 direction. Definition (14) also implies that the critical points of the stationary distribution are characterized by the conditions

$$\frac{g_1(n_1, n_2)}{r_1(n_1 + 1, n_2)} = \frac{g_2(n_1, n_2)}{r_2(n_1, n_2 + 1)} = 1 \quad (15)$$

and coincides with the critical points of the MM equations. In figure 2 we plot the stationary distributions

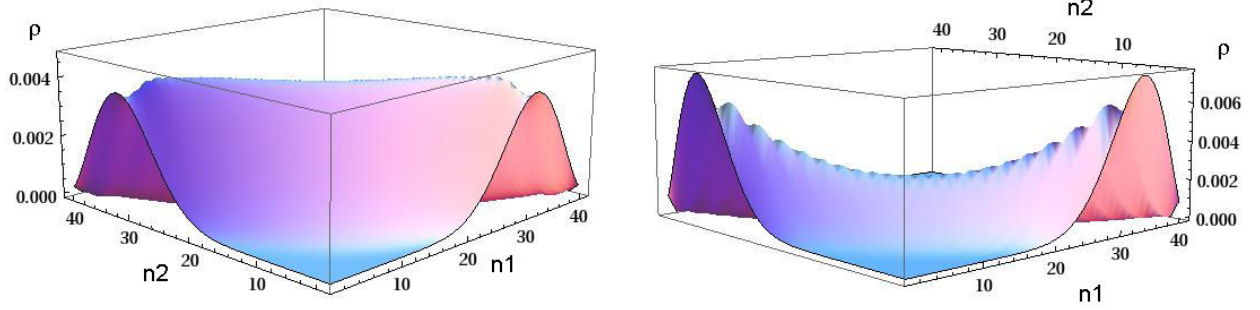


FIG. 2. Stationary distributions for the A and A_P^P states in the double phosphorylation cycle when detailed balance (13) holds with $K_{M_1} = K_{M_4} = 1$ and $K_{M_2} = K_{M_3} = 2$. In the top figure we set the reaction velocities $v_{M_1} = v_{M_2} = 1$ and $v_{M_2} = v_{M_3} = 1.05$ (symmetric case), whereas in the bottom figure we increase the v_{M_2} and v_{M_3} value to 1.15. The number of molecules is $N_T = 40$. The transition from a unimodal distribution to a bimodal distribution is clearly visible.

(14) in the case $N_T = 40$ with $K_{M_1} = K_{M_4} = 1$ and $K_{M_2} = K_{M_3} = 2$; we consider two symmetric cases: $v_{M_1} = v_{M_2} = 1$ with $v_{M_2} = v_{M_3} = 1.05$ or $v_{M_2} = v_{M_3} = 1.15$ (all the units are arbitrary). In the first case, the probability distribution is unimodal, whereas in the second case the transition to a bimodal distribution is observed. Indeed, the system has a phase transition at $v_{M_2} = v_{M_3} \simeq 1.1$ that corresponds to a bifurcation of the critical point defined by the condition (15).

In figure 2 we distinguish two regions: a drift dominated region and a diffusion dominated region. In the first region the chemical reactions mainly follow the gradient of the potential $V(n_1, n_2)$ and tend to concentrate around the stable critical points, so that the dynamic is well described by a Liouville equation[5]. In the second region the drift field (11) is small and the fluctuations due to the finite size introduce a diffusive behaviour. Then the distribution can be approximated by the solution of a Fokker-Planck equation[18]. As discussed in the Appendix, the diffusion region is approximately determined by the conditions

$$\begin{aligned} g_1(n_1, n_2) - r_1(n_1 + 1, n_2) &\simeq g_2(n_1, n_2) - r_2(n_1, n_2 + 1) \\ &\simeq O(1/N_T) \end{aligned} \quad (16)$$

To illustrate this phenomenon, we outline in fig. 3 the region where condition (16) is satisfied (i.e. the gradient of the potential $V(n_1, n_2)$ is close to 0 (12)). This is the region where the fixed points of the MM equation are located, and comparison with fig. 2 shows that it defines the support of the stationary distribution.

In the diffusion dominated region a molecule can undergo a transition from the dephosphorylated equilibrium to the double phosphorylated one. At the stationary state the transition probability from one equilibrium to the other can be estimated by the Fokker-Planck ap-

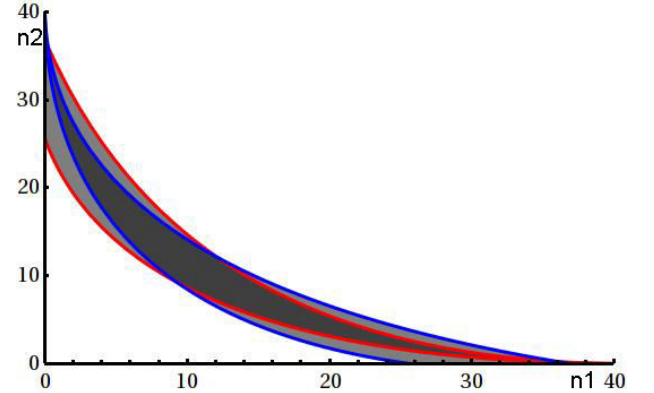


FIG. 3. In grey we show the region where components of the vector field (11) are $\simeq 1$ using the parameter values of fig. 2 (bottom). The blue lines enclose the region where the first component is nearby 1, whereas the red ones enclose the corresponding region for the second component.

proximation; but this does not imply that the Fokker-Planck equation allows us to describe the transient relaxation process toward the stationary state. Indeed, due to the singularity of the thermodynamics limit, the dynamics of transient states may depend critically on finite size effects not described by using the Fokker-Planck approximation[19]. To cope with these problems in the PdPC model further studies are necessary.

When the current (8) is not zero the CME (2) relaxes toward a Non-Equilibrium Stationary State (NESS) and the field (11) is not conservative. We shall perform a perturbative approach to point out the effects of currents on the NESS nearby the transition region to the bimodal regime. We consider the following decomposition for the

vector field

$$\begin{aligned}\ln a_1(n_1, n_2) &= -D_1 V_0(n_1, n_2) + D_2 H(n_1, n_2) \\ \ln a_2(n_1, n_2) &= -D_2 V_0(n_1, n_2) - D_1 H(n_1, n_2) \\ n_1 + n_2 &\leq N_T - 1\end{aligned}\quad (17)$$

where the rotor potential $H(n_1, n_2)$ takes into account the irreversible rotational part. The potential $H(n_1, n_2)$ can be recursively computed using the discrete Laplace equation

$$(D_1 D_1 + D_2 D_2)H = D_2 \ln(a_1(n_1, n_2)) - D_1 \ln(a_2(n_1, n_2)) \quad (18)$$

where $n_1 + n_2 \leq N_T - 2$, with the boundary conditions $H(n, N - n) = H(n, N - 1 - n) = 0$ (see Appendix). Assuming that the potential H is small with respect to V , we can approximate the NESS by using the Maxwell-Boltzmann distribution (14) with $V = V_0$. However, as we shall show in the next section, at the phase transition even the effect of small currents becomes critical, and the study of higher perturbative orders is necessary. To point out the relation among the rotor potential H , the NESS ρ_s and the chemical flux J , we compute the first perturbative order letting $\rho_s(n_1, n_2) = \exp(-V_0(n_1, n_2) - V_1(n_1, n_2))$. From definition (5) the condition (8) reads:

$$\begin{aligned}&\exp(-V_0(n_1, n_2) - V_1(n_1, n_2)) r_1(n_1, n_2) \\ &\cdot (\exp(D_2 H(n_1 - 1, n_2)) - \exp(-D_1 V_1(n_1 - 1, n_2))) \\ &= D_2 a(n_1, n_2) \\ &\exp(-V_0(n_1, n_2) - V_1(n_1, n_2)) r_2(n_1, n_2) \\ &\cdot (\exp(-D_1 H(n_1, n_2 - 1)) - \exp(-D_2 V_1(n_1, n_2 - 1))) \\ &= -D_1 a(n_1, n_2)\end{aligned}\quad (19)$$

$V_1(n_1, n_2)$ turns out to be an effective potential that simulates the current's effect on the unperturbed stationary distribution by using a conservative force. We note that if the rotor potential H is zero, then both the current potential A and the potential correction V_1 are zero, so that all these quantities are of the same perturbative order, and the first perturbative order of eqs. (19) reads

$$\begin{aligned}&\exp(-V_0(n_1, n_2)) r_1(n_1, n_2) \cdot \\ &(D_1 V_1(n_1 - 1, n_2) + D_2 H(n_1 - 1, n_2)) = D_2 A(n_1, n_2) \\ &\exp(-V_0(n_1, n_2)) r_2(n_1, n_2) \cdot \\ &(D_2 V_1(n_1, n_2 - 1) - D_1 H(n_1, n_2 - 1)) = -D_1 A(n_1, n_2)\end{aligned}\quad (20)$$

From the previous equations we see that the currents depend both on the rotor potential H and the potential correction V_1 , which is unknown; thus they cannot be directly computed from (20). We obtain an equation for

V_1 by eliminating the potential A from (20)

$$\begin{aligned}&D_1 (\exp(-V_0(n_1, n_2)) r_1(n_1, n_2) D_1 V_1(n_1 - 1, n_2)) \\ &+ D_2 (\exp(-V_0(n_1, n_2)) r_2(n_1, n_2) D_2 V_1(n_1, n_2 - 1)) = \\ &D_2 (\exp(-V_0(n_1, n_2)) r_2(n_1, n_2) D_1 H(n_1, n_2 - 1)) \\ &- D_1 (\exp(-V_0(n_1, n_2)) r_1(n_1, n_2) D_2 H(n_1 - 1, n_2))\end{aligned}\quad (21)$$

Eq. (21) is defined for $n_1 \geq 1$, $n_2 \geq 1$ and $n_1 + n_2 \leq N_T - 1$, and we can solve the system by introducing the boundary conditions $V_1(n, 0) = V_1(0, n) = V_1(n, N_T - n) = 0$. It is interesting to analyze equation (21) in the phase transition regime. When the recombination terms r_1, r_2 are almost equal and their variation is small (for our parameter choice this is true for $n_1 \simeq n_2$ and $n_1 + n_2 \gg 1$) the r.h.s. can be approximated by

$$\begin{aligned}&\exp(-V_0(n_1, n_2)) [D_2 V_0(n_1, n_2) D_1 H(n_1, n_2 - 1) \\ &- D_1 V_0(n_1, n_2) D_2 H(n_1 - 1, n_2)]\end{aligned}\quad (22)$$

As a consequence, in the transition regime this term is negligible since both $D_1 V_0(n_1, n_2)$ and $D_2 V_0(n_1, n_2)$ tend toward zero in the diffusion region where the bifurcation occurs; thus the first perturbative order is not enough to compute the stationary distribution correction, but higher orders should be considered.

IV. NUMERICAL SIMULATIONS

In order to study the non equilibrium stationary conditions in the double phosphorylation cycle (1) we have perturbed the detailed balance conditions considered in figure (2) by changing the value of the MM constants K_{M_2} and K_{M_3} . In figure (4) we show the rotor field of the potential H in the cases $K_{M_2} = K_{M_3} = 2.2$ and $K_{M_2} = K_{M_3} = 1.8$ when the detailed balance condition (13) does not hold. We see that in the first case (left picture) the rotor field tends to move the particles from the borders toward the central region $n_1 = n_2$, so that we expect an increase of the probability distribution in the center, whereas in the second case the rotor field is directed from the central region to the borders and we expect a decrease of the probability distribution in this region. To illustrate the effect of the potential H , we compare the zero-order approximation of the probability distribution (14), where the potential $V_0(n_1, n_2)$ is computed using decomposition (17) with the stationary solution of the CME (2). The main parameter values are reported in table IV

Case	K_{M_2}	K_{M_3}	v_{M_2}	v_{M_3}
I	1.8	1.8	1.05	1.05
II	1.8	1.8	1.15	1.15
III	2.2	2.2	1.05	1.05
IV	2.2	2.2	1.15	1.15

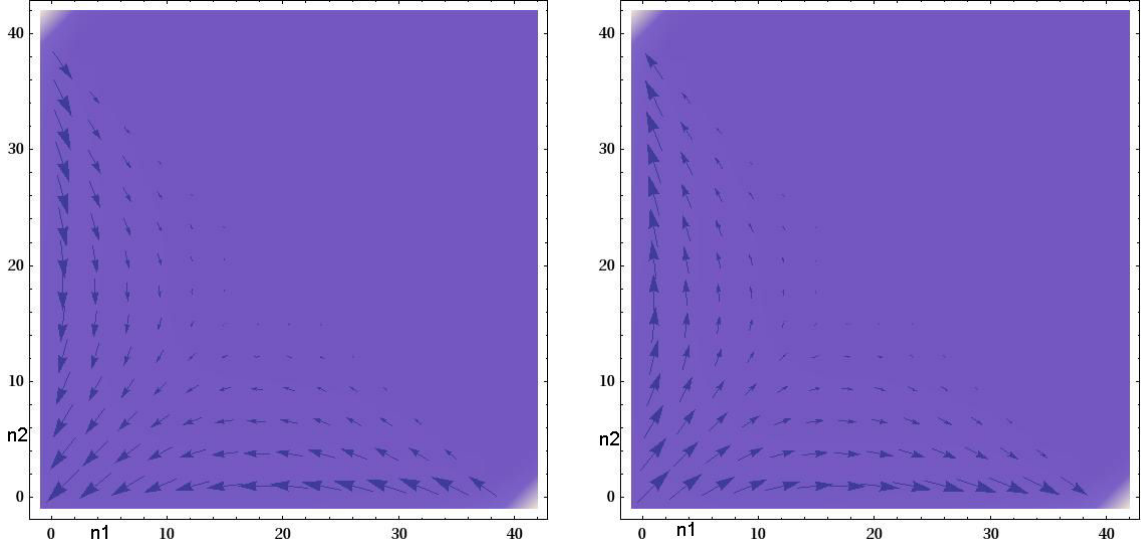


FIG. 4. Plot of the rotor field for the potential H using the following parameter values: $v_{M1} = v_{M2} = 1$, $v_{M2} = v_{M3} = 1.15$ $K_{M1} = K_{M4} = 1$, $N_T = 40$ and $K_{M2} = K_{M3} = 1.8$ (left picture) or $K_{M2} = K_{M3} = 2.2$ (right picture).

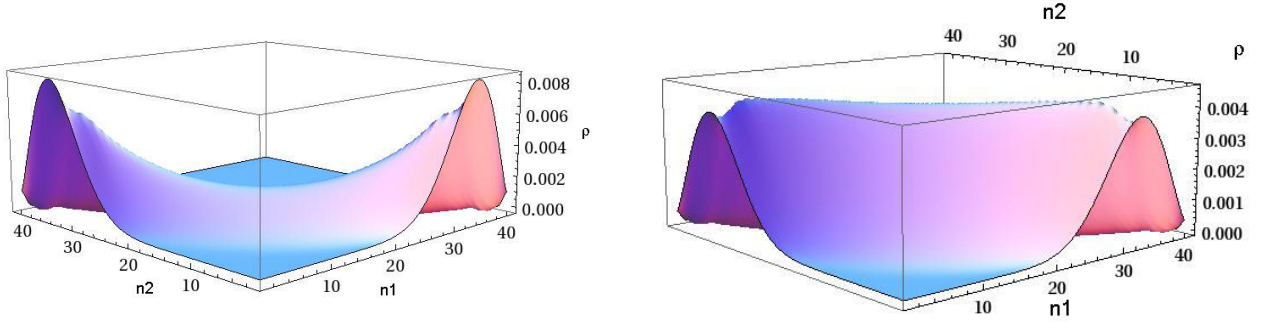


FIG. 5. (Left picture): plot of the zero-order approximation for the probability distribution using the decomposition (17) for the vector field associated to the CME. (Right picture): plot of the stationary distribution computed by directly solving the CME (2). We use the parameter values of the case I in the table IV.

whereas the other parameters values are: $v_{M1} = v_{M2} = 1$, $K_{M1} = K_{M4} = 1$ and $N_T = 40$. For the first parameter set, the zero-order approximation is a bimodal distribution, but the effect of the currents induced by the rotor potential H (see fig. 4) left) are able to destroy the bimodal behaviour by depressing the two maximal at the border (see fig. 5).

If we increase the value of the K_{2M} and K_{3M} (case II), the exact stationary distribution becomes bimodal and the effect of currents is to introduce a strong transition probability between the two distribution maxima (fig. 6).

Therefore, when the MM constants K_{M2} and K_{M3} are < 2 (we note that for $K_{M2} = K_{M3} = 2$, the detailed balance holds), the non-conservative nature of the field (17) introduces a delay in the phase transition from a

mono-modal to a bimodal distribution. However, when we consider $K_{M2} = K_{M3} > 2$ (cases III and IV) the rotor potential H moves the particle towards the borders and the central part of the distribution is depressed. This is shown in the figure 7 where we compare the zero-order approximation of the stationary distribution and the solution of the CME using the case III parameters of the table IV.

Finally, in case IV of table IV, the CME stationary solution undergoes a transition to a bimodal distribution, whereas the zero-order approximation is still mono-modal (fig. 9), so that the effect of currents is to anticipate the phase transition.

Using the stationary solution one can also compute the currents according to definition (5). In figure (9) we plot

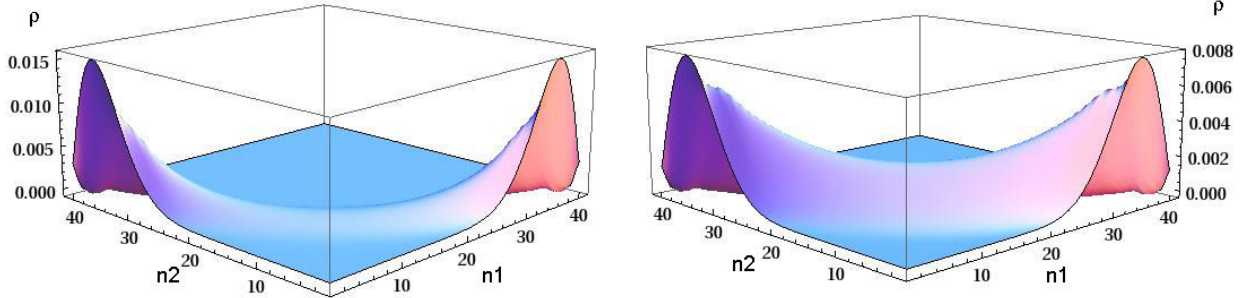


FIG. 6. The same as in fig. 5 using parameter values of case II in table IV.

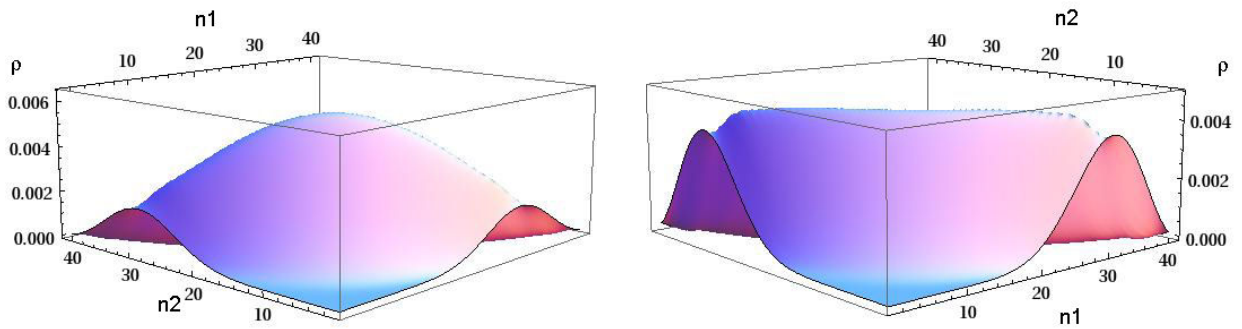


FIG. 7. The same as in fig. 5 using the parameter values of the case III in table IV.

the current vector in case IV parameters to show that the current tends to become normal to the distribution gradient near the maximal value.

This result can be also understood using the perturbative approach (21), where one shows that the main effect of the V_1 potential correction is to compensate the rotor field of H along the distribution gradient directions. As a consequence, the current is zero at the maximal distribution value and condition (15) defines the critical points of the stationary distribution even in the non-conservative case.

V. CONCLUSIONS

The CME approach we present here is a powerful method for studying complex cellular processes, even with significant simplifications such as spatial homogeneity of volumes where the chemical reactions are taking place. The CME theory is attractive for a variety of reasons, including the richness of aspects (the capabil-

ity of coping with fluctuations and chemical fluxes) and the possibility of developing thermodynamics, starting from the distribution function. The violation of detailed balance gives information on the “openness” of the system and on the nature of the bistable regimes, which are induced by the external environment; in contrast, it is a free-energy equilibrium when detailed balance holds. This statement can be expressed in a more rigorous form by introducing the vector field generated from the ratio between the generation and recombination terms, by decomposing it into a sum of “conservative” and “rotational” fields (Helmholtz decomposition) and by relating the chemical fluxes to the non-conservative field. The magnitude of deviations from detailed balance influences the form of the stationary distribution at the transition to a bistable regime, which may be driven by the currents. An interesting test for the prediction of the PdPC CME model would be to perform one experiment with the parameter values chosen to satisfy DB and compare it with another set of parameters where DB is not fulfilled. Our results show that the PdPC can operate across these two

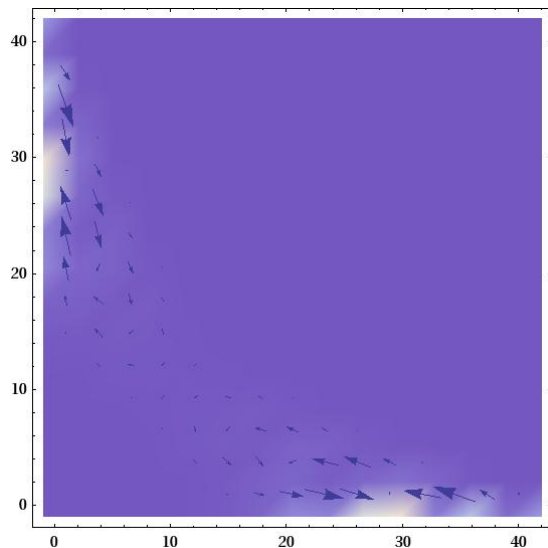


FIG. 8. Current vector field computed by using definition (5) and the stationary solution of the CME with case IV parameters. The distribution is bimodal (cfr. figure 8) and the current lines tend to be orthogonal to the distribution gradient near the maximal value.

regions, and that the transition regime can be explained by the role of the currents, that, within a thermodynamic framework, can be interpreted as the effect of an external energy source. A full thermodynamic analysis of this cycle is beyond the scope of this paper, but we can surmise that this approach might be extended to other cycles in order to quantify if, and how much energy, is required to maintain or create bistability. Another way to extend this analysis would be the generalization to n -step phospho/dephosphorylation cycles, where the stationary distribution will be the product of n independent one-dimensional distributions. In conclusion, our results could be important for a deeper characterization of biochemical signaling cycles that are the molecular basis for complex cellular behaviors implemented as a “switch” between states.

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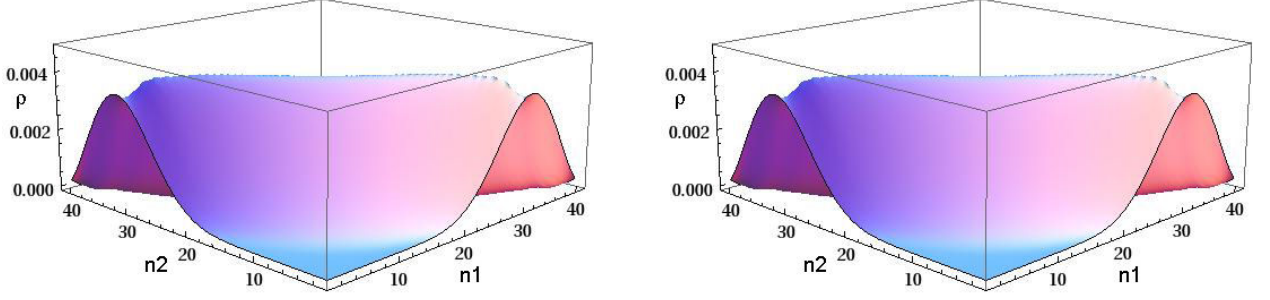


FIG. 9. The same as in fig. 5 using parameter values of case IV in table IV.

VI. APPENDIX

The master equation describes the evolution of one-step Markov Processes according to

$$\begin{aligned} \frac{\partial \rho}{\partial t}(n_1, n_2, t) = & \\ & g_1(n_1 - 1, n_2)\rho(n_1 - 1, n_2, t) - g_1(n_1, n_2)\rho(n_1, n_2, t) \\ & + r_1(n_1 + 1, n_2)\rho(n_1 + 1, n_2, t) - r_1(n_1, n_2)\rho(n_1, n_2, t) \\ & + g_2(n_1, n_2 - 1)\rho(n_1, n_2 - 1, t) - g_2(n_1, n_2)\rho(n_1, n_2, t) \\ & + r_2(n_1, n_2 + 1)\rho(n_1, n_2 + 1, t) - r_2(n_1, n_2)\rho(n_1, n_2, t) \end{aligned} \quad (23)$$

with the boundary conditions for the coefficients

$$\begin{aligned} g_1(n, N - n) = g_2(n, N - n) = 0 \quad \text{and} \\ r_2(n, 0) = r_1(0, n) = 0 \quad n \in [0, N_T] \end{aligned} \quad (24)$$

so that $n_1 + n_2 \leq N_T$. By introducing the difference operators (7), eq. (23) can be written in the form of a continuity equation

$$\frac{\partial \rho}{\partial t}(n_1, n_2, t) = -D_1 J_1(n_1, n_2, t) - D_2 J_2(n_1, n_2, t) \quad (25)$$

where we introduce the current vector J of components:

$$\begin{aligned} J_1(n_1, n_2, t) = & g_1(n_1 - 1, n_2)\rho(n_1 - 1, n_2, t) \\ & - r_1(n_1, n_2)\rho(n_1, n_2, t) \\ J_2(n_1, n_2, t) = & g_2(n_1, n_2 - 1)\rho(n_1, n_2 - 1, t) \\ & - r_2(n_1, n_2)\rho(n_1, n_2, t) \end{aligned} \quad (26)$$

The stationary solution $\rho_s(n_1, n_2)$ is characterized by the zero divergence condition for the current (25). Detailed

balance holds when the current is zero and ρ_s satisfies

$$\begin{aligned} r_1(n_1, n_2)\rho_s(n_1 - 1, n_2) \\ \cdot \left(\frac{\rho_s(n_1, n_2)}{\rho_s(n_1 - 1, n_2)} - \frac{g_1(n_1 - 1, n_2)}{r_1(n_1, n_2)} \right) = 0 \\ r_2(n_1, n_2)\rho_s(n_1, n_2 - 1) \\ \cdot \left(\frac{\rho_s(n_1, n_2)}{\rho_s(n_1, n_2 - 1)} - \frac{g_2(n_1, n_2 - 1)}{r_2(n_1, n_2)} \right) = 0 \end{aligned} \quad (27)$$

for $0 < n_1$ and $0 < n_2$. The previous equations can be written in the form

$$\begin{aligned} D_1 \ln(\rho_s(n_1 - 1, n_2)) = \ln \left(\frac{g_1(n_1 - 1, n_2)}{r_1(n_1, n_2)} \right) \\ D_2 \ln(\rho_s(n_1, n_2 - 1)) = \ln \left(\frac{g_2(n_1, n_2 - 1)}{r_2(n_1, n_2)} \right) \end{aligned} \quad (28)$$

and, if one introduces the the vector field

$$\begin{aligned} a_1(n_1, n_2) = \frac{g_1(n_1, n_2)}{r_1(n_1 + 1, n_2)} \\ a_2(n_1, n_2) = \frac{g_2(n_1, n_2)}{r_2(n_1, n_2 + 1)} \end{aligned} \quad (29)$$

due to the commutative property of the difference operators D_i , detailed balance implies an irrotational character for the vector field $\ln(a(n_1, n_2))$

$$D_2 \ln(a_1(n_1, n_2)) - D_1 \ln(a_2(n_1, n_2)) = 0 \quad (30)$$

If we have no singularities in the domain, eq. (30) is a sufficient condition for the existence of a potential $V(n_1, n_2)$ (cfr. eq. (12)) and the distribution $\rho_s(n_1, n_2)$ can be computed using the recurrence relations

$$\begin{aligned} \rho_s(n_1 + 1, n_2) = a_1(n_1, n_2)\rho_s(n_1, n_2) \\ \rho_s(n_1, n_2 + 1) = a_2(n_1, n_2)\rho_s(n_1, n_2) \end{aligned} \quad (31)$$

Therefore, the components $a_1(n_1, n_2)$ and $a_2(n_1, n_2)$ can also be interpreted as creation operators according to relations (31) and detailed balance condition (30) is equivalent to the commutativity property for these operators. The stationary distribution can be written in the Maxwell-Boltzmann form (14) and the potential $V(n_1, n_2)$ is associated with an “energy function”. We finally observe that the critical points of the stationary distribution ρ_s are defined by the condition

$$a_1(n_1, n_2) = a_2(n_1, n_2) = 1 \quad (32)$$

For the double phosphorylation cycle (1) it is possible to derive explicit expressions for the stationary distribution

$\rho_s(n_1, n_2)$ by applying recursively the relations (31) in a specific order: for example, first moving along the n_2 direction and then along n_1 , we obtain an expression for $\rho_s(n_1, n_2)$ as a function of $\rho_s(0, 0)$

$$\rho_s(n_1, n_2) = \prod_{i=1}^{n_1} \frac{g_1(i-1, n_2)}{r_1(i, n_2)} \prod_{l=1}^{n_2} \frac{g_2(0, l-1)}{r_2(0, l)} \rho_s(0, 0) \quad (33)$$

A direct substitution of the coefficients (3) in the relation (33) gives

$$\begin{aligned} \rho_s(n_1, n_2) &= \prod_{i=1}^{n_1} \frac{K_{M4}V_{M2}(N_T - i - n_2 + 1)}{K_{M2}K_{M4} + K_{M4}(N_T - i - n_2 + 1) + K_{M2}n_2} \cdot \frac{K_{M1}K_{M3} + K_{M1}(N_T - i - n_2) + K_{M3}i}{K_{M3}V_{M1}i} \\ &\cdot \prod_{l=1}^{n_2} \frac{K_{M1}V_{M3}(N_T - l + 1)}{K_{M1}K_{M3} + K_{M1}(N_T - l + 1)} \frac{K_{M2}K_{M4} + K_{M4}(N_T - l) + K_{M2}l}{K_{M2}V_{M4}l} \rho_s(00) \end{aligned}$$

We can further simplify this expression by using the definition of multinomial coefficients and the rising and falling factorial symbols, defined as $x^{(n)} = x(x+1)(x+2)\cdots(x+n-1) = \frac{(x+n-1)!}{(x-1)!}$ and $x_{(n)} = x(x-1)(x-2)\cdots(x-n+1) = \frac{x!}{(x-n)!}$ respectively.

$$\begin{aligned} \rho_s(n_1, n_2) &= \left(\frac{V_{M2}}{V_{M1}}\right)^{n_1} \left(\frac{V_{M3}}{V_{M4}}\right)^{n_2} \binom{N_T - n_2}{n_1} \binom{N_T}{n_2} \\ &\left(\frac{K_{M3} - K_{M1}}{K_{M3}}\right)^{n_1} \left(\frac{K_{M2} - K_{M4}}{K_{M2}}\right)^{n_2} \cdot \\ &\frac{(K_{M1}(1 + n_2 - K_{M3} - N_T) - K_{M3})^{(n_1)}}{(K_{M1} - K_{M3})^{(n_1)}(K_{M2}(1 + \frac{n_2}{K_{M4}}) + N_T - n_2)^{(n_1)}} \cdot \\ &\frac{(K_{M2}(1 + K_{M4}) + K_{M4}(N_T - 1))^{(n_2)}}{(K_{M2} - K_{M4})^{(n_2)}(K_{M3} + N_T)^{(n_2)}} \rho_s(0, 0) \end{aligned}$$

Finally, it is interesting to go to a continuous limit that is equivalent to $N_T \rightarrow \infty$. First we introduce the population densities $A = n_1/N_T$ and $A_P^P = n_2/N_T$ and use the fact that the generation and recombination rates are invariant by substituting n_1 and n_2 with A and A_P^P .

Then we approximate

$$\begin{aligned} D_1 V(A, A_P^P) &= V(A + 1/N_T, A_P^P) - V(A, A_P^P) \\ &= \frac{1}{N_T} \frac{\partial V}{\partial A}(A + \frac{1}{2N_T}, A_P^P) + O(1/N_T^3) \end{aligned}$$

and a similar expression holds for $D_2 V(A, A_P^P)$. According to eq. (12), the partial derivatives of $V(A, A_P^P)$ are bounded when $N_T \rightarrow \infty$ only in the domain where the following approximation holds (diffusion dominated region)

$$\frac{g_i(A, A_P^P)}{r_i(A, A_P^P)} = 1 + O(1/N_T) \quad i = 1, 2 \quad (34)$$

and we can estimate

$$\begin{aligned} &\ln \left(\frac{g_1(A, A_P^P)}{r_1(A + 1/N_T, A_P^P)} \right) \\ &\simeq -2 \frac{r_1(A + 1/N_T, A_P^P) - g_1(A, A_P^P)}{r_1(A + 1/N_T, A_P^P) + g_1(A, A_P^P)} + O(1/N_T^3) \\ &\ln \left(\frac{g_2(A, A_P^P)}{r_2(A, A_P^P + 1/N_T)} \right) \\ &\simeq -2 \frac{r_2(A, A_P^P + 1/N_T) - g_2(A, A_P^P)}{r_2(A, A_P^P + 1/N_T) + g_2(A, A_P^P)} + O(1/N_T^3) \end{aligned} \quad (35)$$

Then we may approximate (we use the convention of leaving out the dependence on A_P^P)

$$\frac{r_1(A + 1/N_T) - g_1(A)}{r_1(A + 1/N_T) + g_1(A)} = \frac{r_1(A + 1/2N_T) - g_1(A + 1/2N_T) + 1/2N_T (\partial r_1/\partial A(A + 1/2N_T) + \partial g_1/\partial A(A + 1/2N_T))}{r_1(A + 1/N_T) + g_1(A)} \quad (36)$$

up to an error of order $O(1/N_T^2)$ (a similar expression is obtained for the second equation). Therefore, detailed balance in the continuous limit reads

$$\begin{aligned} \frac{r_1(A, A_P^P) - g_1(A, A_P^P) + 1/2N_T (\partial r_1/\partial A(A, A_P^P) + \partial g_1/\partial A(A, A_P^P))}{r_1(A + 1/2N_T, A_P^P) + g_1(A - 1/2N_T, A_P^P)} &= -\frac{1}{2N_T} \frac{\partial V}{\partial A}(A, A_P^P) \\ \frac{r_2(A, A_P^P) - g_2(A, A_P^P) + 1/2N_T (\partial r_2/\partial A_P^P(A, A_P^P) + \partial g_2/\partial A_P^P(A, A_P^P))}{r_2(A, A_P^P + 1/2N_T) + g_2(A, A_P^P - 1/2N_T)} &= -\frac{1}{2N_T} \frac{\partial V}{\partial A_P^P}(A, A_P^P) \end{aligned} \quad (37)$$

The limit $N_T \rightarrow \infty$ turns out to be singular since

$$\begin{aligned} -\frac{\partial V}{\partial A}(A, A_P^P) &= \frac{2N_T(r_1(A, A_P^P) - g_1(A, A_P^P)) + \partial r_1/\partial A(A, A_P^P) + \partial g_1/\partial A(A, A_P^P)}{r_1(A, A_P^P) + g_1(A, A_P^P)} \\ -\frac{\partial V}{\partial A_P^P}(A, A_P^P) &= \frac{2N_T(r_2(A, A_P^P) - g_2(A, A_P^P)) + \partial r_2/\partial A_P^P(A, A_P^P) + \partial g_2/\partial A_P^P(A, A_P^P)}{r_2(A, A_P^P) + g_2(A, A_P^P)} \end{aligned} \quad (38)$$

Hence in the diffusion domain defined by condition (34), we recover detailed balance for a Fokker-Planck equation with drift and diffusion coefficients defined as:

$$c_i(A, A_P^P) = 2N_T (r_i(A, A_P^P) - g_i(A, A_P^P)) \quad i = 1, 2$$

and

$$b_i(A, A_P^P) = r_i(A, A_P^P) + g_i(A, A_P^P) \quad i = 1, 2$$

In the diffusion region the drift and the diffusion coefficients are of the same order, otherwise $c_i(A, A_P^P) \gg b_i(A, A_P^P)$ when $N_T \gg 1$. As a consequence, the stationary solution of F.P. equation is an approximation of the stationary distribution of the CME in the diffusion region, but the approximation of the transient state dynamics using the F.P. equation requires further studies due to the singularity of the thermodynamic limit.

In the generic case of eq. (23), we represent the r.h.s. of eq. (28) as a sum of a rotational and a gradient vector

fields

$$\begin{aligned} \ln a_1(n_1, n_2) &= D_1 V(n_1, n_2) + D_2 H(n_1, n_2) \\ \ln a_2(n_1, n_2) &= D_2 V(n_1, n_2) - D_1 H(n_1, n_2) \end{aligned} \quad (39)$$

Taking into account the condition $n_1 + n_2 \leq N_T - 1$, from the eqs. (39) we get the discrete Poisson equations

$$\begin{aligned} (D_1 D_1 + D_2 D_2) V(n_1, n_2) &= \\ D_1 \ln(a_1(n_1, n_2)) + D_2 \ln(a_2(n_1, n_2)) &= \\ (D_1 D_1 + D_2 D_2) H(n_1, n_2) &= \\ D_2 \ln(a_1(n_1, n_2)) - D_1 \ln(a_2(n_1, n_2)) &= \end{aligned} \quad (40)$$

We remark that the r.h.s. of eqs. (40) is defined only if $n_1 + n_2 \leq N - 2$ and corresponds to $N(N - 1)/2$ independent equations, whereas we have $(N + 2)(N + 1)/2$ unknown values $H(n_1, n_2)$. As a consequence from the explicit form of the discrete Poisson operator

$$(D_1^2 + D_2^2)H(n_1, n_2) = H(n_1 + 2, n_2) - 2H(n_1 + 1, n_2) + H(n_1, n_2) + H(n_1, n_2 + 2) - 2H(n_1, n_2 + 1) + H(n_1, n_2)$$

we can set the boundary conditions $H(n, N - n) = H(n, N - 1 - n) = 0$ and recursively solve the system

setting

$$\begin{aligned} 2H(n, N - 2 - n) &= \\ \ln \left(\frac{a_1(n, N - 1 - n)a_2(n, N - 2 - n)}{a_1(n, N - 2 - n)a_2(n + 1, N - 2 - n)} \right) &= \end{aligned} \quad (41)$$

and successively using the equations

$$2H(n, N-j-n) = -H(n+2, n-j-n) - H(n, N-j-n-2) + 2H(n+1, N-j-n) + 2H(n, N-j-n-1) + \ln \frac{a_1(n, N-j-n+1)a_2(n, N-j-n)}{a_1(n, N-j-n)a_2(n+1, N-j-n)} \quad (42)$$

for $N \geq j > 2$. Once $H(n_1, n_2)$ is computed, we define the “potential” $V(n_1, n_2)$ by using eq. (39). The recursion relations (42) can be written in an exponential form by defining

$$R(n_1, n_2) = \exp(H(n_1, n_2))$$

$$\begin{aligned} R(n, N-j-n) = & \sqrt{\frac{a_1(n, N-j-n+1)a_2(n, N-j-n)}{a_1(n, N-j-n)a_2(n+1, N-j-n)}} \\ & \cdot \frac{R(n+1, N-j-n)R(n, N-j-n-1)}{\sqrt{R(n+2, n-j-n)R(n, N-j-n-2)}} \end{aligned} \quad (43)$$

As a consequence, the recurrence (31) reads

$$\begin{aligned} \rho_s(n_1+1, n_2) &= a_1(n_1, n_2) \frac{R(n_1, n_2)}{R(n_1, n_2+1)} \rho_s(n_1, n_2) \\ \rho_s(n_1, n_2+1) &= a_2(n_1, n_2) \frac{R(n_1+1, n_2)}{R(n_1, n_2)} \rho_s(n_1, n_2) \end{aligned} \quad (44)$$

for all $n_1 + n_2 \leq N-1$.

The current components (25) turn out to be proportional to the rotational part of the field (39) (i.e. to $H(n_1, n_2)$) [11], so that the current vanishes at the points where condition (32) is satisfied. One can prove that the critical points of the stationary distribution are still defined by eq. (32). Indeed, if one computes the formal expansion of the generation and recombination rates

around a solution of eqs. (31)

$$\begin{aligned} g(n) &= 1 + \frac{\partial g}{\partial n}(n^*) \cdot \Delta n + \dots \\ r(n) &= 1 + \frac{\partial r}{\partial n}(n^*) \cdot \Delta n + \dots \end{aligned} \quad (45)$$

(for the sake of simplicity we have normalized the value of the generation and recombination rate to 1 at the critical point) the current components can be approximated by the expressions

$$\begin{aligned} J_1^s(n_1, n_2) &\simeq \rho_s(n_1-1, n_2) - \rho_s(n_1, n_2) \\ &+ \frac{\partial g_1}{\partial n}(n^*) \cdot \Delta n \rho_s(n_1-1, n_2) - \frac{\partial r_1}{\partial n}(n^*) \cdot \Delta n \rho_s(n_1, n_2) \\ J_2^s(n_1, n_2) &\simeq \rho_s(n_1, n_2-1) - \rho_s(n_1, n_2) \\ &+ \frac{\partial g_2}{\partial n}(n^*) \cdot \Delta n \rho_s(n_1, n_2-1) - \frac{\partial r_2}{\partial n}(n^*) \cdot \Delta n \rho_s(n_1, n_2) \end{aligned} \quad (46)$$

At the critical point n_* we get

$$\rho_s(n_1^*-1, n_2^*) = \rho_s(n_1^*, n_2^*-1) = \rho_s(n_1^*, n_2^*) \quad (47)$$

since $J^s(n_1^*, n_2^*) = 0$. The condition (47) means the n^* is a critical point for the stationary distribution ρ_s .

When $H(n_1, n_2)$ is small, the detailed balance solution (14) is a good approximation of the stationary solution $\rho_s(n_1, n_2)$ and a perturbative approach can be applied. Let us write the stationary condition (6) in the form

$$\begin{aligned} D_1 r_1(n_1, n_2) \rho_s(n_1, n_2) \left(1 - a_1(n_1-1, n_2) \frac{\rho_s(n_1-1, n_2)}{\rho_s(n_1, n_2)} \right) + \\ D_2 r_2(n_1, n_2) \rho_s(n_1, n_2) \left(1 - a_2(n_1, n_2-1) \frac{\rho_s(n_1, n_2-1)}{\rho_s(n_1, n_2)} \right) = 0 \end{aligned} \quad (48)$$

By using the definitions (39), we assume that the rotational field is associated with a potential $\epsilon H(n_1, n_2)$, with $\epsilon \ll 1$ perturbation parameter and we write the stationary solution in the form

$$\rho_s(n_1, n_2) = C \exp(V(n_1, n_2) + \epsilon V_1(n_1, n_2)) \quad (49)$$

From a direct calculation we get

$$\begin{aligned}
& D_1 r_1(n_1, n_2) e^{V(n_1, n_2)} (1 - \exp(\epsilon(D_2 H(n_1 - 1, n_2) - D_1 V_1(n_1 - 1, n_2)))) \\
& + D_2 r_2(n_1, n_2) e^{V(n_1, n_2)} (1 - \exp(-(\epsilon D_1 H(n_1, n_2 - 1) + D_2 V_1(n_1, n_2 - 1)))) \simeq \\
& \quad \epsilon D_1 r_1(n_1, n_2) e^{V(n_1, n_2)} (D_1 V_1(n_1 - 1, n_2) - D_2 H(n_1 - 1, n_2)) \\
& \quad + \epsilon D_2 r_2(n_1, n_2) e^{V(n_1, n_2)} (D_2 V_1(n_1, n_2 - 1) + D_1 H(n_1, n_2 - 1)) = 0
\end{aligned} \tag{50}$$

for all the values $n_1 + n_2 \leq N - 1$ and $n_i \geq 1$. The correction potential $V_1(n_1, n_2)$ has to be computed from

the previous equation, and it enters in the definition of the stationary currents.